

# Pharmacodynamic Activity of Telithromycin at Simulated Clinically Achievable Free-Drug Concentrations in Serum and Epithelial Lining Fluid against Efflux (*mefE*)-Producing Macrolide-Resistant *Streptococcus pneumoniae* for Which Telithromycin MICs Vary

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The present study, using an *in vitro* model, assessed telithromycin pharmacodynamic activity at simulated clinically achievable free-drug concentrations in serum (S) and epithelial lining fluid (ELF) against efflux (*mefE*)-producing macrolide-resistant *Streptococcus pneumoniae*. Two macrolide-susceptible (PCR negative for both *mefE* and *ermB*) and 11 efflux-producing macrolide-resistant [PCR-positive for *mefE* and negative for *ermB*] *S. pneumoniae* strains with various telithromycin MICs (0.015 to 1 µg/ml) were tested. The steady-state pharmacokinetics of telithromycin were modeled, simulating a dosage of 800 mg orally once daily administered at time 0 and at 24 h (free-drug maximum concentration [ $C_{max}$ ] in serum, 0.7 µg/ml; half-life [ $t_{1/2}$ ], 10 h; free-drug  $C_{max}$  in ELF, 6.0 µg/ml;  $t_{1/2}$ , 10 h). Starting inocula were  $10^6$  CFU/ml in Mueller-Hinton Broth with 2% lysed horse blood. Sampling at 0, 2, 4, 6, 12, 24, and 48 h assessed the extent of bacterial killing (decrease in  $\log_{10}$  CFU/ml versus initial inoculum). Free-telithromycin concentrations in serum achieved in the model were  $C_{max}$   $0.9 \pm 0.08$  µg/ml, area under the curve to MIC ( $AUC_{0-24\text{ h}}$ )  $6.4 \pm 1.5$  µg · h/ml, and  $t_{1/2}$  of  $10.6 \pm 0.6$  h. Telithromycin-free ELF concentrations achieved in the model were  $C_{max}$   $6.6 \pm 0.8$  µg/ml,  $AUC_{0-24\text{ h}}$   $45.5 \pm 5.5$  µg · h/ml, and  $t_{1/2}$  of  $10.5 \pm 1.7$  h. Free-telithromycin S and ELF concentrations rapidly eradicated efflux-producing macrolide-resistant *S. pneumoniae* with telithromycin MICs up to and including 0.25 µg/ml and 1 µg/ml, respectively. Free-telithromycin S and ELF concentrations simulating  $C_{max}/MIC \geq 3.5$  and  $AUC_{0-24\text{ h}}/MIC \geq 25$  completely eradicated ( $\geq 4 \log_{10}$  killing) macrolide-resistant *S. pneumoniae* at 24 and 48 h. Free-telithromycin concentrations in serum simulating  $C_{max}/MIC \geq 1.8$  and  $AUC_{0-24\text{ h}}/MIC \geq 12.5$  were bacteriostatic (0.1 to 0.2  $\log_{10}$  killing) against macrolide-resistant *S. pneumoniae* at 24 and 48 h. In conclusion, free-telithromycin concentrations in serum and ELF simulating  $C_{max}/MIC \geq 3.5$  and  $AUC_{0-24\text{ h}}/MIC \geq 25$  completely eradicated ( $\geq 4 \log_{10}$  killing) macrolide-resistant *S. pneumoniae* at 24 and 48 h.

Macrolide (azithromycin, clarithromycin, and erythromycin) resistance in *Streptococcus pneumoniae* is presently ~25% in the United States and approximately 13% in Canada (1, 3, 28, 31). Macrolide resistance in *S. pneumoniae* involves alteration of the ribosomal target site or production and utilization of an efflux mechanism (6, 9, 29, 33). The production of ribosomal methylase, which alters the ribosomal target site of the macrolide, is usually coded for by the *ermB* gene and confers broad macrolide, lincosamide, and streptogramin B resistance (6, 9, 29, 33). The second mechanism, which results in macrolide efflux, is coded by the *mefA* or *mefE* genes (6, 9, 29, 33). Efflux is macrolide specific (14- and 15-membered macrolides only) and does not affect the lincosamide or streptogramins (28, 32). Note that *ermB*-positive *S. pneumoniae* strains generally exhibit high-level ( $MIC_{90} \geq 64$  µg/ml) macrolide resistance, while *mefA*- or *mefE*-positive *S. pneumoniae* strains exhibit low- to moderate-level resistance ( $MIC_{90}$  4 µg/ml) (6, 9, 29,

33). Both of these mechanisms are transmissible to other isolates (6, 9, 29, 33). Presently, in North America, *mefE*-positive *S. pneumoniae* is more common than *ermB*-positive *S. pneumoniae* and *mefE* strains make up the majority of macrolide-resistant *S. pneumoniae* strains (6, 9). In many European countries, *ermB*-positive *S. pneumoniae* strains are more prevalent (28, 32).

Although reports associating macrolide-resistant *S. pneumoniae* with macrolide clinical failure in the treatment of community-acquired respiratory infections are available, they are not that common (24).

Ketolides are a new class of semisynthetic agents derived from erythromycin A and are designed specifically to combat respiratory tract pathogens that have acquired resistance to macrolides (5, 7, 8, 11, 22, 26, 32). The main structural difference between ketolides and the macrolides is the lack of L-cladinose sugar at position 3 of the erythronolide A ring and its replacement with a 3-keto group (28, 33). Telithromycin and cethromycin (formerly ABT-773) have excellent *in vitro* activity against many pathogens causing community-acquired respiratory infections, including penicillin and macrolide-resistant strains (5–9, 22, 26, 28, 32). Ketolides demonstrate potent

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TABLE 1. Telithromycin susceptibilities of macrolide-susceptible and macrolide-resistant *mefE* *S. pneumoniae*

Isolate	Result					
	<i>mefE</i>	<i>ermB</i>	Azithromycin MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>	Clarithromycin MIC ( $\mu\text{g/ml}$ ) <sup>b</sup>	Clindamycin MIC ( $\mu\text{g/ml}$ ) <sup>c</sup>	Telithromycin MIC ( $\mu\text{g/ml}$ ) <sup>d</sup>
11771	–	–	0.06	0.03	$\leq 0.12$	0.008
11888	–	–	0.06	0.03	$\leq 0.12$	0.008
12629	+	–	8	4	$\leq 0.12$	0.015
35168	+	–	2	1	$\leq 0.12$	0.03
8086	+	–	8	4	$\leq 0.12$	0.06
16218	+	–	8	1	$\leq 0.12$	0.06
11183	+	–	2	1	$\leq 0.12$	0.12
18701	+	–	8	4	$\leq 0.12$	0.12
17258	+	–	8	4	$\leq 0.12$	0.25
1333	+	–	8	8	$\leq 0.12$	0.5
UK185	+	–	16	8	$\leq 0.12$	0.5
3543	+	–	16	8	$\leq 0.12$	1
1217	+	–	16	16	$\leq 0.12$	1

<sup>a</sup> Susceptible,  $\leq 0.5$   $\mu\text{g/ml}$ ; intermediate, 1.0  $\mu\text{g/ml}$ ; resistant,  $\geq 2$   $\mu\text{g/ml}$ .

<sup>b</sup> Susceptible,  $\leq 0.25$   $\mu\text{g/ml}$ ; intermediate, 0.5  $\mu\text{g/ml}$ ; resistant,  $\geq 1$   $\mu\text{g/ml}$ .

<sup>c</sup> Susceptible,  $\leq 0.25$   $\mu\text{g/ml}$ ; intermediate, 0.5  $\mu\text{g/ml}$ ; resistant,  $\geq 1$   $\mu\text{g/ml}$ .

<sup>d</sup> Susceptible,  $\leq 1$   $\mu\text{g/ml}$ ; intermediate, 2  $\mu\text{g/ml}$ ; resistant,  $\geq 4$   $\mu\text{g/ml}$  (17, 31, 33).

activity against most macrolide-resistant streptococci, including *ermB*- and *mefA*- or *mefE*-positive *Streptococcus pneumoniae* (5–9, 22, 26, 28, 32). Their pharmacokinetics display a long half-life ( $t_{1/2}$ ) as well as extensive tissue distribution and uptake into respiratory tissues and fluids, allowing for once-daily (OD) dosing (4, 12, 14, 15, 16, 19, 27). Presently only limited data are available on the pharmacodynamic activity of ketolides against macrolide-resistant *S. pneumoniae* in comparison to macrolides (13, 34).

The purpose of this study was to assess the pharmacodynamic activity of the ketolide telithromycin at simulated clinically achievable free-drug concentrations in serum (S) and epithelial lining fluid (ELF) against efflux-producing *mefE* macrolide-resistant *S. pneumoniae*.

#### MATERIALS AND METHODS

**Bacterial strains and culture conditions.** Two macrolide-susceptible and 11 efflux-producing *mefE* macrolide-resistant strains of *S. pneumoniae* were evaluated (Table 1). As the *mef* gene in *S. pneumoniae* occurs as two variants, discrimination between *mefA* and *mefE* was performed by PCR-restriction fragment length polymorphism analysis according to a previously described protocol (2). Isolates were obtained from the Canadian Respiratory Organism Susceptibility Study (CROSS) (31). Telithromycin and azithromycin MICs are depicted in Table 1. The wild-type strains 11771 and 11888 were PCR-negative for *mefA*, *mefE*, and *ermB* and were macrolide-susceptible (azithromycin MIC  $\leq 0.5$   $\mu\text{g/ml}$ ). Macrolide-resistant (azithromycin MIC  $\geq 2$   $\mu\text{g/ml}$ ) strains were PCR-positive for *mefE* and PCR-negative for *ermB* (Table 1). Isolates were chosen to represent a variety of telithromycin MICs (0.015 to 1  $\mu\text{g/ml}$ ). The method and conditions used for PCR detection of *mefE* and *ermB* genotypes have been previously described (9).

**Antibiotic preparation and susceptibility testing.** Antibiotics were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were prepared, and dilutions were made according to previously described methods (17). Following two subcultures from frozen stock, antibiotic MICs were determined by the NCCLS broth microdilution method (17, 18). All MIC determinations were performed in triplicate on separate days.

**In vitro pharmacodynamic model.** The in vitro pharmacodynamic model used in this study has been previously described (21). Logarithmic phase cultures were prepared using a 0.5 McFarland ( $10^8$  CFU/ml) standard by suspending several colonies in cation-supplemented Mueller-Hinton broth with 2% lysed horse blood (Oxoid, Nepean, Ontario, Canada) (pH 7.1). This suspension was diluted 1:100, and 20  $\mu\text{l}$  of the diluted suspension was further diluted in 60 ml of cation-supplemented Mueller-Hinton broth with 2% lysed horse blood. The

resulting suspension was allowed to grow overnight at 35°C in ambient air (21, 30, 34). After a maximum of 17 h, the suspension was further diluted to 1:10 and 60 ml of the diluted suspension was added to the in vitro pharmacodynamic model. Viable bacterial counts consistently yielded a starting inoculum of approximately  $10^6$  CFU/ml (21, 30, 34). This final inoculum was introduced into the central compartment (volume, 610 ml) of the in vitro pharmacodynamic model.

**Pharmacokinetics and pharmacodynamics simulated.** Telithromycin was modeled based upon data obtained from previous publications (our target or simulated concentrations), simulating steady-state pharmacokinetics after a dosage of 800 mg orally (p.o.) OD (4, 12, 16, 28, 32). Thus, if after the administration of telithromycin at 800 mg, the maximum serum concentration ( $C_{\text{max}}$ ) was  $\sim 2.2$   $\mu\text{g/ml}$  (and the serum protein binding was  $\sim 70\%$ ) (4, 28, 32), it was assumed that the free  $C_{\text{max}}$  in serum was  $\sim 0.7$   $\mu\text{g/ml}$ . Thus, in serum (S) we simulated the maximum concentration [ $C_{\text{max}}$ ] at 0.7  $\mu\text{g/ml}$ ,  $t_{1/2}$  10 h. For epithelial lining fluid (ELF), it has been reported that the  $C_{\text{max}}$  of telithromycin after 800 mg is  $\sim 15$   $\mu\text{g/ml}$  (12). As the protein binding of telithromycin in ELF was not known, it was assumed to be similar to that of serum (70%) and thus only the likely concentration of free drug in ELF ( $C_{\text{max}} \sim 6.0$   $\mu\text{g/ml}$ ) was simulated. Not knowing what the exact  $t_{1/2}$  of telithromycin was in ELF, we chose to simulate a  $t_{1/2}$  for telithromycin of  $\sim 10$  h for both serum and ELF and to simulate a slightly higher free-drug concentration in ELF ( $C_{\text{max}} \sim 6.0$   $\mu\text{g/ml}$ ) knowing this would result in a larger  $\text{AUC}_{0-24 \text{ h}}$ . Telithromycin was administered once at time 0 and as a second dose at 24 h. Thus, two doses were administered every 24 h for 48 h. Pharmacodynamic experiments were performed in ambient air at 37°C. Samples were collected at 0, 1, 2, 4, 6, 12, 24, and 48 h for both pharmacokinetic and pharmacodynamic assessment (21, 30, 34). Telithromycin concentrations in the pharmacodynamic model were determined microbiologically with a bioassay (21, 30, 34). Actual or achieved telithromycin concentrations were determined in quadruplicate using *Bacillus subtilis* ATCC 6633 as the test organism with lower limits of quantification of 0.03  $\mu\text{g/ml}$ . The plates were incubated aerobically for 18 h at 37°C. Concentrations were determined in relation to the diameters of the inhibition zones caused by the known concentrations from the standard series. The correlation coefficient of this assay was 0.80. Intra- and interrun variability of quality control samples were  $\leq 6.5\%$  and  $\leq 5.8\%$ , respectively. The actual or achieved concentrations of telithromycin and not the target or simulated concentrations were used in pharmacodynamic interpretations (e.g.,  $C_{\text{max}}/\text{MIC}$  and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ ). Pharmacodynamic parameters of  $C_{\text{max}}/\text{MIC}$ ,  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ , and time above the MIC ( $T > \text{MIC}$ ) were derived from the actual or achieved telithromycin concentrations obtained in the model relative to the MIC of the strain in question.

Pharmacodynamic sampling was performed over 48 h with viable bacterial counts assessed by plating serial 10-fold dilutions onto cation-supplemented Mueller-Hinton agar with 2.0% lysed horse blood. Plates were incubated for 24 h at 37°C in ambient air. The lowest dilution plated was 0.1 ml of undiluted sample, and the lowest level of detection was 200 CFU/ml ( $2.0 \log_{10}$ ) (21, 30, 34).

TABLE 2. Simulated (target) and achieved (actual) telithromycin pharmacokinetics

Pharmacokinetic parameter	Result <sup>a</sup>			
	Simulated (serum)	Achieved (Serum)	Simulated (ELF) <sup>b</sup>	Achieved (ELF)
$C_{max}$ (µg/ml) ± SD	0.7	0.9 ± 0.08	6.0	6.6 ± 0.8
$AUC_{0-24 h}$ (µg · h/ml) ± SD	4.5	6.4 ± 1.5	38.6	45.5 ± 5.5
$t_{1/2}$ (h) ± SD	10	10.6 ± 1.6	10	10.5 ± 1.7

<sup>a</sup> Target (simulated) and actual (achieved) pharmacokinetic parameters of telithromycin after simulating a dosage of 800 mg PO OD.

<sup>b</sup> ELF, epithelial lining fluid.

RESULTS

Table 1 shows the MICs of telithromycin and azithromycin against the clinical isolates utilized in this study. Strains were chosen to include macrolide-susceptible (wild-type) as well as low-level (MIC 2 to 4 µg/ml), intermediate (MIC 8 µg/ml), and high-level (MIC 16 µg/ml) macrolide-resistant *mefE* strains and *ermB*-positive *S. pneumoniae*. As well, isolates were chosen to represent a wide distribution of telithromycin MICs ranging from 0.008 µg/ml to 1 µg/ml. As shown in Table 1, all *mefE* strains were susceptible to clindamycin.

**Pharmacokinetics.** Target (simulated) and actual (achieved) pharmacokinetic parameters of telithromycin after simulating a dosage of 800 mg p.o. OD (free serum and free epithelial lining fluid) achieved in the model were similar (Table 2). Target (simulated) and actual (achieved) pharmacokinetic parameters of telithromycin achieved in serum were as follows: free drug  $C_{max}$ , 0.7 µg/ml (occurring at  $t = 0$ );  $AUC_{0-24 h}$ , 4.5 µg · h/ml;  $t_{1/2}$ , 10 h; and  $C_{max}$ , 0.9 ± 0.08 (± standard deviation [SD]) µg/ml (occurring at  $t = 0$ );  $AUC_{0-24 h}$ , 6.4 ± 1.5 (± SD) µg · h/ml;  $t_{1/2}$ , 10.6 ± 0.6 (± SD) h, respectively. Telithromycin target (simulated) and actual (achieved) pharmacokinetic parameters achieved under free-drug conditions in ELF were  $C_{ELF-free}$  maximum, 6.0 µg/ml (occurring at  $t = 0$ );  $AUC_{0-24 h}$ , 38.6 µg · h/ml;  $t_{1/2}$ , 10 h; and  $C_{ELF-free}$  maximum, 6.6 ± 0.8 (± SD) µg/ml (occurring at  $t = 0$ );  $AUC_{0-24 h}$ , 45.5 ± 5.5 (± SD) µg·h/ml;  $t_{1/2}$ , 10.5 ± 1.7 (± SD) h, respectively.

**Pharmacodynamics.** Table 3 describes the killing of *S. pneu-*

*moniae* with achieved telithromycin free-drug concentrations in serum. Free-telithromycin concentrations in serum resulted in bactericidal ( $\geq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) activity as early as 4 h for strains with telithromycin MICs  $\leq 0.12$  µg/ml (Table 3). This bactericidal activity was maintained for the entire 48 h of the experimental period. For strain 17258 with a telithromycin MIC of 0.25 µg/ml, free-telithromycin concentrations in serum were bacteriostatic ( $\leq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) for the first 12 h followed by complete bacterial eradication ( $\geq 4.0 \log_{10}$  CFU/ml decrease versus initial inoculum) at 24 and 48 h (Table 3). Free-telithromycin concentrations in serum resulted in bacteriostatic ( $\leq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) activity over the entire 48 h period for strains with telithromycin MIC 0.5 µg/ml (Table 3). For strains with telithromycin MIC of 1 µg/ml, free-telithromycin concentrations in serum were bacteriostatic ( $\leq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) over the first 6 to 12 h followed by rapid regrowth at 24 and 48 h (Table 3).

Table 3 also describes the killing of *S. pneumoniae* with achieved free-telithromycin concentrations in epithelial lining fluid (ELF). Free-telithromycin concentrations in ELF resulted in bactericidal ( $\geq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) activity as early as 2 h for strains with telithromycin MICs  $\leq 0.12$  µg/ml (Table 3). This bactericidal activity was maintained for the entire 48 h of the experimental period. For strains with telithromycin MICs of 0.25 µg/ml and 0.5 µg/ml, free-telithromycin concentrations in ELF were bacteriostatic ( $\leq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) for the first 6 h followed by complete bacterial eradication ( $\geq 4.0 \log_{10}$  CFU/ml decrease versus initial inoculum) at 12, 24, and 48 h (Table 3). For strains with telithromycin MICs of 1 µg/ml, free-telithromycin concentrations in ELF were bacteriostatic ( $\leq 3.0 \log_{10}$  CFU/ml decrease versus initial inoculum) for the first 4 to 6 h followed by complete bacterial eradication ( $\geq 4.0 \log_{10}$  CFU/ml decrease versus initial inoculum) at 12 to 24 h (Table 3).

The pharmacodynamic parameters associated with bacterial inhibition (decrease in  $\log_{10}$  CFU/ml at 24 h versus initial inoculum) by telithromycin at simulated achieved free-drug concentrations in serum as well as in ELF are depicted in

TABLE 3. Telithromycin killing of *S. pneumoniae* at simulated free-drug concentrations in serum and epithelial lining fluid

Strain (MIC [µg/ml])	Mean $\log_{10}$ cfu/ml killing at indicated h (serum result/epithelial lining fluid result) <sup>a</sup>					
	2	4	6	12	24	48
11771 (0.008)	1.9/3.1	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
11888 (0.008)	1.7/3.3	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
12629 (0.015)	1.7/3.1	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
35168 (0.03)	1.1/3.0	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
8086 (0.06)	1.0/3.0	3.2/ $\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
16218 (0.06)	0.9/3.0	3.0/ $\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
11183 (0.12)	0.9/3.0	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
18701 (0.12)	1.1/3.2	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
17258 (0.25)	0.5/0.2	0.6/1.2	1.6/2.0	2.0/ $\geq 4.0$	$\geq 4.0/\geq 4.0$	$\geq 4.0/\geq 4.0$
1333 (0.5)	0.1/0.2	0.2/1.0	0.4/2.1	1.0/ $\geq 4.0$	0.2/ $\geq 4.0$	0.1/ $\geq 4.0$
UK185 (0.5)	0/0.2	0.1/1.1	0.3/1.9	1.2/ $\geq 4.0$	0.1/ $\geq 4.0$	0.1/ $\geq 4.0$
3543 (1)	0/0.3	0/0.7	0.4/1.0	0/3.2	0/ $\geq 4.0$	0/ $\geq 4.0$
1217 (1)	0/0.5	0.2/1.7	0.4/3.0	0.1/ $\geq 4.0$	0/ $\geq 4.0$	0/ $\geq 4.0$

<sup>a</sup> Growth reduction relative to initial inoculum. Growth controls started at  $10^6$  cfu/ml, reached  $10^8$  cfu/ml at 6 hours, and maintained this inoculum over the 48-hour study period.

TABLE 4. Pharmacodynamics of telithromycin versus macrolide-susceptible and macrolide-resistant *S. pneumoniae* ( $T > MIC$ )<sup>a</sup>

Isolate/MIC ( $\mu\text{g/ml}$ )	Free-drug result in:			
	Serum		ELF	
	T > MIC (% of dosing interval)	Outcome at 24 h <sup>b</sup>	T > MIC (% of dosing interval)	Outcome at 24 h
11771/0.008	100	E <sup>c</sup>	100	E
11888/0.008	100	E	100	E
12629/0.015	100	E	100	E
35168/0.03	100	E	100	E
8086/0.06	100	E	100	E
16218/0.06	100	E	100	E
11183/0.12	100	E	100	E
18701/0.12	100	E	100	E
17258/0.25	84	E	100	E
1333/0.5	42	0.2	100	E
UK185/0.5	42	0.1	100	E
3543/1	0	0	100	E
1217/1	0	0	100	E

<sup>a</sup> Assumption made that protein binding in ELF was same as in serum (fraction unbound, 0.30).

<sup>b</sup> Log<sub>10</sub> killing at 24 hours (0 represents regrowth relative to the initial inoculum).

<sup>c</sup> E, eradicated.

Tables 4, 5, and 6. Free-telithromycin concentrations in serum and ELF simulating  $C_{\text{max}}/MIC \geq 3.5$  and  $AUC_{0-24\text{ h}}/MIC \geq 25$  (time above the MIC [ $T > MIC$ ] of 84%, shown for comparative purposes only) completely eradicated ( $\geq 4 \log_{10}$  killing) macrolide-resistant *S. pneumoniae* at 24 and 48 h. Free-telithromycin concentrations in serum simulating  $C_{\text{max}}/MIC \geq 1.8$  and  $AUC_{0-24\text{ h}}/MIC \geq 12.5$  (time above the MIC [ $T > MIC$ ] of 42%, shown for comparative purposes only) were bacteriostatic (0.1 to 0.2 log<sub>10</sub> killing) against macrolide-resistant *S. pneumoniae* at 24 and 48 h. Free-telithromycin concentrations in serum simulating  $C_{\text{max}}/MIC \leq 0.9$  and  $AUC_{0-24\text{ h}}/MIC \leq 6.3$  (time above the MIC [ $T > MIC$ ] of 0%, shown for

TABLE 5. Pharmacodynamics of telithromycin versus macrolide-susceptible and macrolide-resistant *S. pneumoniae* ( $C_{\text{max}}/MIC$ )<sup>a</sup>

Isolate/MIC ( $\mu\text{g/ml}$ )	Free-drug result in:			
	Serum		ELF	
	$C_{\text{max}}/MIC$	Outcome <sup>b</sup>	$C_{\text{max}}/MIC$	Outcome
11771/0.008	113	E <sup>c</sup>	825	E
11888/0.008	113	E	825	E
12629/0.015	56.3	E	413	E
35168/0.03	28.1	E	206	E
8086/0.06	14.1	E	103	E
16218/0.06	14.1	E	103	E
11183/0.12	7.0	E	51.6	E
18701/0.12	7.0	E	51.6	E
17258/0.25	3.5	E	25.8	E
1333/0.5	1.8	0.2	12.9	E
UK185/0.5	1.8	0.1	12.9	E
3543/1	0.9	0	6.4	E
1217/1	0.9	0	6.4	E

<sup>a</sup> Assumption made that protein binding in ELF was same as in serum (fraction unbound, 0.30).

<sup>b</sup> Log<sub>10</sub> killing at 24 hours (0 represents regrowth relative to the initial inoculum).

<sup>c</sup> E, eradicated.

TABLE 6. Pharmacodynamics of telithromycin vs. macrolide-susceptible and macrolide-resistant *S. pneumoniae* ( $AUC_{0-24\text{ h}}/MIC$ )

Isolate/MIC ( $\mu\text{g/ml}$ )	Free-drug result in:			
	Serum		ELF	
	$AUC_{0-24\text{ h}}/MIC$	Outcome <sup>b</sup>	$AUC_{0-24\text{ h}}/MIC$	Outcome
11771/0.008	800	E <sup>c</sup>	5688	E
11888/0.008	800	E	5688	E
12629/0.015	400	E	2844	E
35168/0.03	200	E	1422	E
8086/0.06	100	E	711	E
16218/0.06	100	E	711	E
11183/0.12	50	E	355	E
18701/0.12	50	E	355	E
17258/0.25	25	E	178	E
1333/0.5	12.5	0.2	89	E
UK185/0.5	12.5	0.1	89	E
3543/1	6.3	0	44	E
1217/1	6.3	0	44	E

<sup>a</sup> Assumption made that protein binding in ELF was same as in serum (fraction unbound, 0.30).

<sup>b</sup> Log<sub>10</sub> killing at 24 hours (0 represents regrowth relative to the initial inoculum).

<sup>c</sup> E, eradicated.

comparative purposes only) resulted in regrowth of macrolide-resistant *S. pneumoniae* at 24 and 48 h.

## DISCUSSION

In a previous study, using this same in vitro model, we assessed telithromycin pharmacodynamic activity (against macrolide-susceptible and macrolide-resistant *S. pneumoniae*) at simulated clinically achievable free-drug concentrations in serum (S) and epithelial lining fluid (ELF) against strains with telithromycin MICs of 0.008 to 0.03  $\mu\text{g/ml}$ . Against these very susceptible isolates, telithromycin serum and epithelial lining fluid concentrations resulted in eradication from the model in 4 h with no regrowth over 48 h (34). The purpose of this study was to assess the pharmacodynamic activity of telithromycin at simulated clinically achievable free-drug concentrations in serum and epithelial lining fluid (ELF) against efflux-producing macrolide-resistant *S. pneumoniae* with various telithromycin MICs (from 0.008 to 1  $\mu\text{g/ml}$ ). In this study, we modeled telithromycin based upon data obtained from previous publications of simulations of steady-state pharmacokinetics after a dosage of 800 mg p.o. OD (4, 12, 16, 28, 32). Thus, if after the administration of telithromycin 800 mg, the maximum concentration ( $C_{\text{max}}$ ) in serum was  $\sim 2.2 \mu\text{g/ml}$  (and the serum protein binding was  $\sim 70\%$ ) (4, 28, 32), it was assumed that the free  $C_{\text{max}}$  in serum was  $\sim 0.7 \mu\text{g/ml}$ . As our pharmacodynamic model contains no high molecular weight protein such as albumin, no protein binding occurs in the model and thus all drug that is added is non-protein-bound or free drug capable of crossing bacterial membranes and exerting a microbiological or pharmacological response. Assuming that, after administration of 800 mg of telithromycin, only the free drug in serum ( $C_{\text{max}}$ , 0.7  $\mu\text{g/ml}$ ) is active and not the entire protein-bound and free drug ( $C_{\text{max}}$ , 2.2  $\mu\text{g/ml}$ ) may underestimate the pharmacodynamic activity of telithromycin in serum. However, we chose in this study to study only the pharmacodynamic poten-



tial of the free drug. Thus, in serum (S) we simulated the maximum concentration at a  $C_{\max}$  of 0.7  $\mu\text{g/ml}$  and a  $t_{1/2}$  of 10 h. For epithelial lining fluid (ELF), it has been reported that the  $C_{\max}$  of telithromycin after 800 mg is  $\sim 15 \mu\text{g/ml}$  (12). As the protein binding of telithromycin in ELF was not known, it was assumed to be similar to that of serum (70%) and thus only the likely concentration of free drug in ELF ( $C_{\max} \sim 6.0 \mu\text{g/ml}$ ) was simulated. We chose to simulate a  $C_{\max}$  in ELF of  $\sim 6.0 \mu\text{g/ml}$  and not 4.5  $\mu\text{g/ml}$  because it has been reported that the  $t_{1/2}$  of telithromycin in ELF is longer than in serum (16). Not knowing what the exact  $t_{1/2}$  of telithromycin was in ELF, we chose to simulate a  $t_{1/2}$  for telithromycin of  $\sim 10$  h for both serum and ELF and to simulate a slightly higher free-drug concentration in ELF ( $C_{\max} \sim 6.0 \mu\text{g/ml}$ ) knowing this would result in a larger  $\text{AUC}_{0-24 \text{ h}}$ . As with serum, it was assumed that only the free drug in the ELF ( $C_{\max} \sim 6.0 \mu\text{g/ml}$ ) is active and not the entire protein-bound and free drug ( $C_{\max} \sim 15 \mu\text{g/ml}$ ). It is true that these assumptions may underestimate the pharmacodynamic activity of telithromycin in ELF; however, we chose in this study only to examine the pharmacodynamic potential of the free drug. It should be mentioned that the exact methods of how best to model ELF concentrations using an in vitro model are under debate.

Using the above-described pharmacodynamic model, we clearly showed that free-telithromycin concentrations in ELF rapidly eradicated macrolide-resistant *S. pneumoniae* with telithromycin MICs ranging from 0.015  $\mu\text{g/ml}$  to 1  $\mu\text{g/ml}$  (Table 3). Free-telithromycin concentrations in serum rapidly eradicated macrolide-resistant *S. pneumoniae* with telithromycin MICs up to and including 0.25  $\mu\text{g/ml}$  (Table 3). Pharmacodynamically, free telithromycin concentrations in serum and ELF simulating  $C_{\max}/\text{MIC} \geq 3.5$  and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC} \geq 25$  (time above the MIC [ $T > \text{MIC}$ ] of 84%) completely eradicated ( $\geq 4 \log_{10}$  killing) macrolide-resistant *S. pneumoniae* at 24 and 48 h (Tables 4, 5, and 6). It should be clear that although the pharmacodynamics of telithromycin correlate with  $C_{\max}/\text{MIC}$  and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ , we also showed the  $T > \text{MIC}$  for comparative purposes only and not to imply that the pharmacodynamics of telithromycin correlate with  $T > \text{MIC}$ .

Comparing the ketolide telithromycin to the macrolide azithromycin, we previously reported that azithromycin serum and epithelial lining fluid concentrations rapidly eradicated macrolide-susceptible *S. pneumoniae* but did not eradicate macrolide-resistant *S. pneumoniae* regardless of resistance phenotype (30). It should, however, be mentioned that our model simulates an immunocompromised host as no component of the immune system is added to the model. Thus, whether in an immunocompetent host, azithromycin can eradicate macrolide-resistant *S. pneumoniae* is not known. As the majority of *S. pneumoniae* in North America are macrolide-susceptible ( $\sim 75\%$  in the United States and  $\sim 87\%$  in Canada), this may help to explain the excellent bacteriological and clinical outcomes obtained with macrolides (such as azithromycin) versus comparator antibiotics in clinical studies of community-acquired respiratory infections, such as community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute sinusitis, and otitis media, where *S. pneumoniae* is a key pathogen (28). However, the rapid and extensive eradication of macrolide-resistant *S. pneumoniae* by telithromycin, when simulating clinically achievable free-drug concentrations in serum

and epithelial lining fluid in this study, suggests that ketolides offer an advantage compared to macrolides such as azithromycin which are not able to eradicate macrolide-resistant *S. pneumoniae* (whether *mefE* or *ermB*) in serum, epithelial lining fluid, or middle ear fluid (30). These differences may help explain why ketolides compared to macrolides may result in reductions in hospitalization rates when treating community-acquired pneumonia (20, 25).

Only limited data are available regarding the pharmacodynamic properties of ketolides (4, 10, 13, 23, 34). Jacobs et al. demonstrated that against gram-positive cocci such as *S. pneumoniae*, telithromycin demonstrated postantibiotic effects of 0.3 to 3.8 h and postantibiotic sub-MIC effects of 0.8 to 4.6 h (10). It has been reported that telithromycin is a concentration-dependent bacterial killer with eradication being related to  $\text{AUC}/\text{MIC}$  and  $C_{\max}/\text{MIC}$  (4, 13, 14, 19, 34). Odenholt et al. reported that against *S. pneumoniae*, telithromycin demonstrated extremely fast ( $\sim 1$  h) bactericidal ( $\geq 3 \log_{10}$  killing) activity with  $C_{\max}/\text{MIC} \geq 37.5$  (23). Kim et al. using a murine pneumococcal pneumonia model reported that free  $C_{\max}/\text{MIC}$  and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$  best explained the relationship between ketolide (cethromycin) drug exposure and reductions in viable bacterial counts (13). These authors documented free-drug  $C_{\max}/\text{MIC}$  of 1 and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$  of 50 as resulting in bacteriostatic effects and maximal survival at free-drug  $C_{\max}/\text{MIC}$  and  $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ s twice these amounts (13). In this study, we also observed very rapid bactericidal activity (within 4 h) against *S. pneumoniae* in simulations of free-telithromycin concentrations in serum and ELF, with pharmacodynamics of  $C_{\max}/\text{MIC} \geq 7$  and area under the curve to MIC ( $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ )  $\geq 50$  (Tables 5 and 6).

In conclusion, telithromycin concentrations in serum and epithelial lining fluid rapidly eradicated efflux-producing macrolide-resistant *S. pneumoniae* with telithromycin MICs up to and including 0.25 and 1  $\mu\text{g/ml}$ , respectively. Free-telithromycin concentrations in serum and ELF simulating  $C_{\max}/\text{MIC} \geq 3.5$  and area under the curve to MIC ( $\text{AUC}_{0-24 \text{ h}}/\text{MIC}$ )  $\geq 25$  completely eradicated ( $\geq 4 \log_{10}$  killing) macrolide-resistant *S. pneumoniae* at 24 and 48 h. Finally, it should once again be mentioned that the exact methods of how best to model in vivo ELF concentrations using an in vitro model are under debate.

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#### REFERENCES

- Butler, J. C., J. Hofmann, M. S. Cetron, J. A. Elliot, R. R. Facklam, and R. F. Breiman. 1996. The continued emergence of drug-resistant *Streptococcus pneumoniae* in the United States: an update from the Centers for Disease Control and Prevention's Pneumococcal Sentinel Surveillance System. *J. Infect. Dis.* 174:986-993.
- Del Grosso, M., F. Iannelli, C. Messina, M. Santagati, N. Petrosillo, S. Stefani, G. Pozzi, and A. Pantosti. 2002. Macrolide efflux genes *mefA* and *mefE* are carried by different genetic elements in *Streptococcus pneumoniae*. *J. Clin. Microbiol.* 40:774-778.
- Doern, G. V., K. P. Heilmann, H. K. Huynh, P. R. Rhomberg, S. L. Coffam, and A. B. Bruuggemann. 2001. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in the United States during 1999-2000, including a comparison of rates since 1994-1995. *Antimicrob. Agents Chemother.* 45:1721-1729.
- Drusano, G. 2001. Pharmacodynamic and pharmacokinetic considerations in antimicrobial selection: Focus on telithromycin. *Clin. Microbiol. Infect.* 7(Suppl. 3):24-29.
- Farrell, D., and D. Felmingham. 2004. Activities of telithromycin against

- 13,874 *Streptococcus pneumoniae* isolates collected between 1999–2003. Antimicrob. Agents Chemother. **48**:1882–1884.
6. Farrell, D. J., and S. G. Jenkins. 2004. Distribution across the USA of macrolide resistance and macrolide resistance mechanism among *Streptococcus pneumoniae* isolates collected from patients with respiratory tract infections: PROTEKT US 2001–2002. J. Antimicrob. Chemother. **54**(Suppl. 1):17–22.
  7. Farrell, D. J., I. Morrissey, S. Bakker, S. Buckridge, and D. Felmingham. 2004. In vitro activities of telithromycin linezolid quinupristin-dalfopristin against *Streptococcus pneumoniae* with macrolide resistance due to ribosomal mutations. Antimicrob. Agents Chemother. **48**:3169–3171.
  8. Hoban, D. J., G. G. Zhanel, and J. A. Karlowsky. 1999. In vitro activity of the novel ketolide HMR 3647 and comparative oral antibiotics against Canadian respiratory tract isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Diagn. Microbiol. Infect. Dis. **35**:37–44.
  9. Hoban, D. J., A. Wierzbowski, K. A. Nichol, and G. G. Zhanel. 2001. Macrolide-resistant *Streptococcus pneumoniae* in Canada from 1998–1999: prevalence of *mefA* and *ermB* and susceptibility to ketolides. Antimicrob. Agents Chemother. **45**:2147–2150.
  10. Jacobs, M. R., S. Bajaksouzian, and P. C. Appelbaum. 2003. Telithromycin post-antibiotic and post-antibiotic sub-MIC effects for 10 gram-positive cocci. J. Antimicrob. Chemother. **52**(5):809–812.
  11. Jorgensen, J. H., S. A. Crawford, M. L. McElmeel, and C. G. Whitney. 2004. Activities of cethromycin and telithromycin against recent North American isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **48**:605–607.
  12. Khair, O. A., J. M. Andrews, D. Honeybourne, G. Jevons, F. Vacheron, and R. Wise. 2001. Lung concentrations of telithromycin after oral dosing. J. Antimicrob. Chemother. **47**:837–840.
  13. Kim, M., W. Zhou, P. R. Tessier, D. Xuan, M. Ye, C. H. Nightingale, and D. P. Nicolau. 2002. Bactericidal effect and pharmacodynamics of cethromycin (ABT-773) in a murine pneumococcal pneumonia model. Antimicrob. Agents Chemother. **46**:3185–3192.
  14. Maglio, D., D. P. Nicolau, and C. H. Nightingale. 2003. Impact of pharmacodynamics on dosing of macrolides, azalides, ketolides. Infect. Dis. Clin. North Am. **17**:563–577.
  15. Muller-Serieys, C., P. Soler, C. Cantalloube, F. Lemaitre, H. P. Gia, F. Brunner, and A. Andreumont. 2001. Bronchopulmonary disposition of the ketolide telithromycin (HMR 3647). Antimicrob. Agents Chemother. **45**:3104–3108.
  16. Muller-Serieys, C., J. Andrews, F. Vacheron, and C. Cantalloube. 2004. Tissue kinetics of telithromycin, the first ketolide antibacterial. J. Antimicrob. Chemother. **53**:149–157.
  17. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5, 5th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
  18. National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial susceptibility testing: supplemental tables. M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
  19. Nicolau, D. P. 2004. Clinical use of antimicrobial pharmacodynamic profiles to optimize treatment outcomes in community-acquired bacterial respiratory tract infections: Applications to telithromycin. Exp. Opin. Pharmacother. **5**:229–235.
  20. Niederman, M. S., J. R. Chang, J. Stewart, R. Nusrat, and R. B. Nieman. 2004. Comparison of hospitalization rates in patients with community acquired pneumonia treated with 10 days of telithromycin or clarithromycin. Curr. Med. Res. Opin. **20**:740–756.
  21. Noreddin, A., D. Roberts, K. Nichol, A. Wierzbowski, D. J. Hoban, and G. G. Zhanel. 2002. Pharmacodynamic modeling of clarithromycin against macrolide-resistant [PCR-positive *mefA*(A) or *ermB*(B)] *Streptococcus pneumoniae* simulating clinically achievable serum and epithelial lining fluid free-drug concentrations. Antimicrob. Agents Chemother. **46**:4029–4034.
  22. Novotny, G. W., L. Jakobsen, N. M. Andrewsen, J. Poehlsgaard, and S. Douthwaite. 2004. Ketolide antimicrobial activity persists after disruption of interactions with domain II of 23S rRNA. Antimicrob. Agents Chemother. **48**:3677–3683.
  23. Odenholt, I., E. Lowdin, and O. Cars. 2001. Pharmacodynamics of telithromycin in vitro against respiratory tract pathogens. Antimicrob. Agents Chemother. **45**:23–29.
  24. Rzeszutek, M., A. Wierzbowski, J. Conly, W. Bishai, D. Hoban, and G. G. Zhanel. 2004. A review of clinical failures involving macrolide-resistant *Streptococcus pneumoniae*. Int. J. Antimicrob. Agents **24**:95–104.
  25. Tellier, G., J. R. Chang, C. V. Asche, B. Lavin, J. Stewart, and S. D. Sullivan. 2004. Comparison of hospitalization rates in patients with community acquired pneumonia treated with telithromycin for 5 or 7 days or with clarithromycin for 10 days. Curr. Med. Res. Opin. **20**:739–747.
  26. Walsh, F., F. Carnegy, J. Willcock, and S. Amyes. 2004. Comparative in vitro activity of telithromycin against macrolide resistant and susceptible *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*. J. Antimicrob. Chemother. **53**:793–796.
  27. Zhanel, G. G. 2001. Influence of pharmacokinetic and pharmacodynamic principles on antibiotic selection. Curr. Infect. Dis. Rep. **3**:29–34.
  28. Zhanel, G. G., M. Dueck, D. J. Hoban, L. Vercaigne, J. Embil, S. S. Gin, and J. A. Karlowsky. 2001. Macrolides and ketolides: a review focusing on respiratory infections. Drugs **61**:443–498.
  29. Zhanel, G. G., and J. A. Karlowsky. 2001. Ribosomal resistance: emerging problems and potential solutions. Curr. Infect. Dis. Rep. **1**:459–463.
  30. Zhanel, G. G., M. DeCorby, A. Noreddin, C. Mendoza, A. Cumming, K. Nichol, A. Wierzbowski, and D. J. Hoban. 2003. Pharmacodynamic activity of azithromycin against macrolide-susceptible and macrolide-resistant (PCR positive *mefA* or *ermB*) *Streptococcus pneumoniae* simulating clinically achievable free serum and epithelial lining fluid (ELF) and middle ear fluid (MEF) concentrations. J. Antimicrob. Chemother. **52**:83–88.
  31. Zhanel, G. G., L. Palatnick, K. Nichol, T. Bellyou, D. Low, and D. J. Hoban. 2003. Antimicrobial resistance in respiratory tract *Streptococcus pneumoniae* isolates: results of the Canadian Respiratory Organism Susceptibility Study, 1997 to 2002. Antimicrob. Agents Chemother. **47**:1867–1874.
  32. Zhanel, G. G., A. Wierzbowski, T. Hisanaga, and D. J. Hoban. 2004. The use of ketolides in the treatment of upper respiratory tract infections. Clin. Infect. Dis. Rep. **6**:191–199.
  33. Zhanel, G. G., T. Hisanaga, K. Nichol, A. Wierzbowski, and D. J. Hoban. 2003. Emerging treatments for macrolide resistant bacteria. Exp. Opin. Emerg. Drugs **8**:297–321.
  34. Zhanel, G. G., C. Johanson, T. Hisanaga, C. Mendoza, N. Laing, A. Noreddin, A. Wierzbowski, and D. J. Hoban. Pharmacodynamic activity of telithromycin against macrolide-susceptible and macrolide-resistant *Streptococcus pneumoniae* simulating clinically achievable free serum and epithelial lining fluid concentrations. J. Antimicrob. Chemother. **54**:1072–1077.